PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 38280-294741

STUDY TITLE:

SCREENING PLASMA AND TUMOR PHARMACOKINETICS (SPTPK) OF BORTEZOMIB IN FEMALE CD1 NU MICE AFTER A SINGLE INTRAPERITONEAL DOSE

SHORT TITLE:	**Bortezomib Screening Plasma Tumor PK (SPTPK)**
TEST ARTICLE:	Bortezomib
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FOR P-PKSR APPROVED USE AND DISTRIBUTION

Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

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1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The total plasma and tumor PK of bortezomib in female CD1 nu/nu mice (Jax Laboratories, aged 8-16 weeks) was assessed after a single intraperitoneal (IP) injection of 1 mg/kg. Bortezomib (LC Labs, Lot BBZ-106, purity >99%, MolWt 384.24) was dissolved in DMSO and further diluted with 0.9% sodium chloride for injection, USP (NS, Baxter) for a final nominal concentration of 0.1 mg/mL in 2% DMSO / 98% NS and a 10 mL/kg injection volume. The IP route was found to be a convenient method, given the intermittent twice weekly dosing required in efficacy studies. Mice were sacrificed using an IACUC-approved method at 10 min, 1, 3, 24, and 72 hr post-dose, with 3 mice per time point. Whole blood was collected with sodium heparin via cardiac puncture, immediately centrifuged to plasma, and stored on dry ice for remainder of study. Mice were then perfused with PBS via the aorta, the MAST 39 rhabdomyosarcoma orthotopic xenografts excised, rinsed with PBS, and placed on dry ice. At the end of the in vivo procedures, all samples were transferred from dry ice and placed at -80 °C until analysis.

1.2 Bioanalysis

Total plasma and tumor homogenate bortezomib concentrations were assessed using a sensitive and specific liquid chromatography, tandem mass spectrometry assay. First, tissue samples were macerated, diluted with a 5:1 volume of ultrapure water, and homogenized with a bead-based technique [1] on a FastPrep-24 system (MP Biomedicals, Santa Ana, CA). Ceramic lysing matrix beads (MP Biomedicals, Ceramic Bead Lysing Matrix, 3 mg per mg of tissue) were added to the microcentrifuge tubes containing tumor samples. Samples were then subjected to three 60 M/S vibratory cycles of 1 min each on the FastPrep-24 system. To prevent over-heating due to friction, samples were placed on wet ice for 5 min between each cycle. The homogenates were then stored at -80 °C until analysis.

Bortezomib (LC Labs, BBZ-106, purity >99%) stock solutions were prepared in 80% methanol / 20% ultrapure water with 0.1% formic acid and used to spike matrix calibrators and quality controls. Plasma and tissue homogenate samples, 25 µL each, were treated with 10 µL of internal standard (bortezomib-d8, Toronto Research Chemicals, 3-GBH-170-2, purity 98%) 50 ng/mL spiking solution and then protein precipitated with 100 µL of acetonitrile with 0.1% formic acid, vortexed vigorously for 1 minute and centrifuged at 4 °C and 13000g for 5 minutes. A 3 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a Shimadzu SIL-20ACXR autosampler. The LC separation was performed using a Phenomenex Luna C8 80Å LC column (4.0 µm, 30 mm x 2.0 mm) maintained at ambient temperature with gradient elution at a flow rate of 0.25 mL/min. The binary mobile phase consisted of 0.1% formic acid in ultra-pure water in reservoir A and 0.1% formic acid in acetonitrile in reservoir B. The initial mobile phase consisted of 30% B with a linear increase to 100% B in 2 minutes. The column was then rinsed for 2.5 minutes at 100% B and then equilibrated at the initial conditions for 2 minutes for a total run time of 6.5 minutes. Under these conditions, the analyte and IS eluted at 2.56 and 2.53 minutes, respectively.

Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 4000 in the positive ESI mode with monitoring of the following mass transitions: bortezomib 367.4 -> 226.2, bortezomib-d8 374.8 -> 234.0.

The experimental bioanalytical runs were all found to be acceptable for the purpose of a singlicate non-GLP, preclinical PK assessment. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 100 ng/mL range, with a correlation coefficient (R) of ≥0.99. Above the calibration range quality control samples were diluted with adequate precision and accuracy. The lower limit of quantitation (LLOQ), defined as a peak area signal-to-noise ratio of 5 or greater verses a matrix blank with IS, was 1 ng/mL for plasma and 6 ng/mL for tissues due to the dilution factor. The intra-run precision and accuracy was < 5.56% CV and 87.1% to 107%, respectively across the matrices.

1.3 Pharmacokinetic (PK) Analysis

The resultant bortezomib concentration-time (Ct) data were grouped by matrix and time point, and manual imputation of data below the lower limit of quantitation (BLOQ) was as follows: IF at any time point ≥ 2/3rds of the Ct results were above the LLOQ, the BLOQ data were replaced with a value of ½ LLOQ, ELSE the entire time point's data were treated as missing. Then, using Phoenix WinNonlin 6.4 (Certara USA, Inc., Princeton, NJ), Ct data summary statistics (arithmetic mean, standard deviation, %CV, minimum, median, maximum) were generated, and the bortezomib arithmetic mean Ct data for each matrix was subjected to noncompartmental pharmacokinetic analysis (NCA). The extravascular model (Model 202) was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" trapezoidal rule. The terminal phase was defined as the two to three time points at the end of the Ct profile, and the elimination rate constant (Ke) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Ke, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + predicted Clast/Ke.

Other NCA parameters estimated included observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of Clast (Tlast), apparent clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The average concentration over a dosing interval (Cavg) was estimated as AUCinf / dosing interval in hours. The apparent partition coefficient of bortezomib from the plasma to the tissue of interest (Kp,*tissue*) was estimated as the ratio of the AUCinf, tissue to AUCinf plasma when available. To estimate a clinically relevant mouse dosage, the resultant mouse plasma AUCinf and Cavg was compared with the reported human plasma PK values at the putative single agent bortezomib maximum tolerated dose at 1.3 mg/m^2 IV on Day 11 of therapy [2]. All inferences were made under the assumption of time-independent, linear and dose-proportional PK in mice and humans.

2.0 RESULTS

Bortezomib concentrations showed rather low variability in the plasma, demonstrating coefficients of variation of 9.66% to 49% across the sampling time points. Tumor penetration appeared to be rapid and extensive, with a Kp,tumor value of ~8 based upon AUClast. As the Ct profile in tumor was virtually flat from 3 to 72 hours, a Ke value could not be estimated and thus was set equal to the plasma Ke value for extrapolated parameter estimation. The apparent T1/2 of bortezomib in the plasma was 111 hr. Apparent tumor half-life was also long with a high Kp value, indicating tumor tissue distribution with high affinity.

In a Phase 1 study of single agent bortezomib administered IV twice weekly, the highest tolerated dose was 1.3 mg/m^2 [2]. The major adverse events with bortezomib are thrombocytopenia and myelosuppression; however, severe peripheral neuropathies and GI events have been observed [3]. Time- and dose-dependent pharmacokinetics have been noted for bortezomib in both mice and humans [2,4]. Thus, we selected Day 11 clinical PK values for comparison, as data at this time point seem to be less influenced by such phenomena. The fraction of bortezomib unbound in plasma (Fu,p) has been reported as moderate and similar across species at approximately 17% [4,5]. While the blood-to-plasma partitioning of bortezomib is appreciable, it's rather similar across species, seemingly prolonged and possibly saturable. Therefore, total plasma AUC values between mice and humans are acceptable for deriving pharmacokinetically equivalent doses for bortezomib. The recommended MED for bortezomib is 0.1 mg/kg IP (in 2% DMSO / 98% NS) twice weekly.

While 0.8 to 1 mg/kg is the most commonly reported bortezomib regimen in mice [6–8], such doses approach the mouse acute LD_{50}, which has been estimated at between 1 to 5 mg/kg [5]. In our experience, such high doses either IP or IV are not well tolerated in non-tumor or tumor bearing CD1 nu or NSG mice for more than 3 weeks. Therefore, while our MED recapitulates human plasma exposures at clinically relevant doses, it also falls within a tolerable and putatively efficacious range for mice. While not as prevalent in the literature, other groups have applied the 0.1 mg/kg or similarly lower doses successfully in their murine models, noting pharmacological effects [8–11].

3.0 REFERENCES

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Mean (SD) Ct Profile of Bortezomib by Group

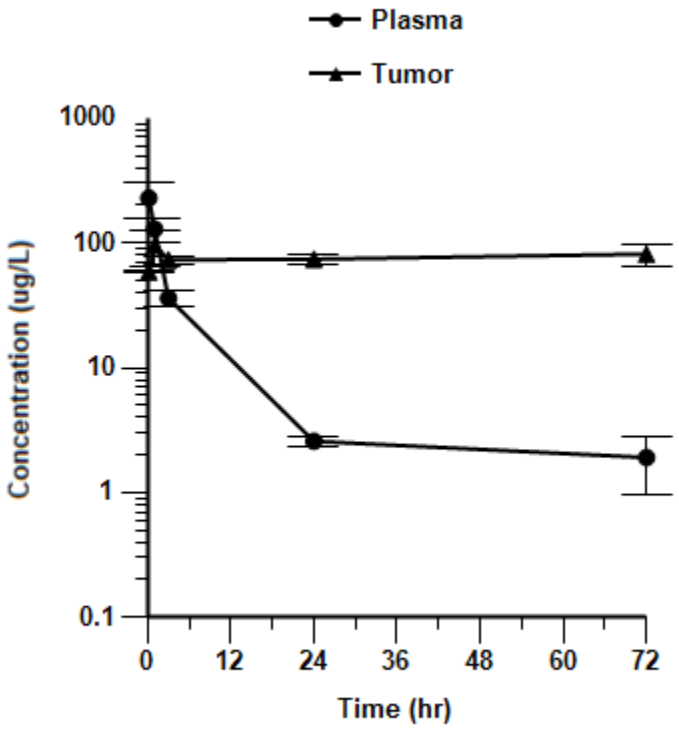

Table 4.1: NCA PK Parameter Estimates of Bortezomib by Group

		Analyte	
		Bortezomib	
		Group	
		Plasma	Tumor
Parameter	**Units**	**Estimate**	
Cmax	ug/L	229	95.3
Tmax	hr	0.167	1.00
AUClast	hr*ug/L	683	5520
AUCinf	hr*ug/L	990	*14200*[b]
Kel	1/hr	0.00624	*0.00624*[a]
T1/2	hr	111	*111*[c]
CL/F	L/hr/kg	1.01	*0.0703*[d]
Vz/F	L/kg	162	11.3
Clast	ug/L	1.91	81.4

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		Analyte	
		Bortezomib	
		Group	
		Plasma	Tumor
Parameter	Units	Estimate	
Tlast	hr	72.0	72.0
Cavg,72 hr	ug/L	13.8	76.6[e]
Kp,tumor	-	-	8.06[f]

a: Ke set equal to that observed in plasma, no terminal elimination in 72 hr period detected
b: Estimated as AUClast + predicted Clast/Ke,plasma
c: Estimated with Ke,plasma as LN(2)/Ke
d: Estimated as derived Dose/AUCinf derived
e: Calculated as AUClast/72 hr
f: Estimated using AUClast values

Table 4.2: Full Summary Statistics of Bortezomib Ct Data by Group

		Analyte	
		Bortezomib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
0.167	N	3	3
	Mean	229	58.7
	SD	72.3	1.17
	Min	177	58.0
	Median	198	58.1
	Max	311	60.0
	CV%	31.6	2.00
	Geometric Mean	222	58.7
	CV% Geometric Mean	30.6	1.99
1.000	N	3	3
	Mean	129	95.3
	SD	28.3	29.7
	Min	103	62.1
	Median	124	105
	Max	159	119
	CV%	21.9	31.1
	Geometric Mean	127	91.9
	CV% Geometric Mean	22.0	35.6
3.000	N	3	3
	Mean	36.1	73.2

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		Analyte	
		Bortezomib	
		Group	
		Plasma	Tumor
Time (hr)		Concentration (ug/L)	
	SD	4.67	5.25
	Min	31.8	67.1
	Median	35.3	76.0
	Max	41.1	76.5
	CV%	12.9	7.17
	Geometric Mean	35.9	73.1
	CV% Geometric Mean	12.9	7.34
24.000	N	3	3
	Mean	2.58	74.1
	SD	0.249	7.77
	Min	2.30	67.7
	Median	2.65	71.9
	Max	2.78	82.7
	CV%	9.66	10.5
	Geometric Mean	2.57	73.8
	CV% Geometric Mean	9.91	10.3
72.000	N	3	3
	Mean	1.91	81.4
	SD	0.937	16.3
	Min	1.28	70.6
	Median	1.46	73.6
	Max	2.99	100
	CV%	49.1	20.0
	Geometric Mean	1.77	80.4
	CV% Geometric Mean	48.0	19.3

Table 4.3: Bortezomib Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Bortezomib	Plasma	0.17	176.82
M1	Bortezomib	Tumor	0.17	60.05
M2	Bortezomib	Plasma	0.17	198.42
M2	Bortezomib	Tumor	0.17	57.97
M3	Bortezomib	Plasma	0.17	311.42

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Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M3	Bortezomib	Tumor	0.17	58.07
M4	Bortezomib	Plasma	1.00	159.37
M4	Bortezomib	Tumor	1.00	104.89
M5	Bortezomib	Plasma	1.00	124.18
M5	Bortezomib	Tumor	1.00	62.05
M6	Bortezomib	Plasma	1.00	103.41
M6	Bortezomib	Tumor	1.00	119.06
M7	Bortezomib	Plasma	3.00	35.34
M7	Bortezomib	Tumor	3.00	75.98
M8	Bortezomib	Plasma	3.00	41.07
M8	Bortezomib	Tumor	3.00	76.46
M9	Bortezomib	Plasma	3.00	31.82
M9	Bortezomib	Tumor	3.00	67.13
M10	Bortezomib	Plasma	24.00	2.65
M10	Bortezomib	Tumor	24.00	71.88
M11	Bortezomib	Plasma	24.00	2.78
M11	Bortezomib	Tumor	24.00	82.73
M12	Bortezomib	Plasma	24.00	2.30
M12	Bortezomib	Tumor	24.00	67.67
M13	Bortezomib	Plasma	72.00	2.99
M13	Bortezomib	Tumor	72.00	70.58
M14	Bortezomib	Plasma	72.00	1.46
M14	Bortezomib	Tumor	72.00	73.58
M15	Bortezomib	Plasma	72.00	1.28
M15	Bortezomib	Tumor	72.00	100.13

Table 4.4: Bortezomib Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Bortezomib	Plasma	0.17	228.89	72.29	3.00
Concentration	ug/L	Bortezomib	Plasma	1.00	128.99	28.29	3.00
Concentration	ug/L	Bortezomib	Plasma	3.00	36.08	4.67	3.00
Concentration	ug/L	Bortezomib	Plasma	24.00	2.58	0.25	3.00
Concentration	ug/L	Bortezomib	Plasma	72.00	1.91	0.94	3.00
Concentration	ug/L	Bortezomib	Tumor	0.17	58.69	1.17	3.00
Concentration	ug/L	Bortezomib	Tumor	1.00	95.33	29.68	3.00
Concentration	ug/L	Bortezomib	Tumor	3.00	73.19	5.25	3.00
Concentration	ug/L	Bortezomib	Tumor	24.00	74.09	7.77	3.00
Concentration	ug/L	Bortezomib	Tumor	72.00	81.43	16.26	3.00

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5.0 ATTACHED FILES

Attached File 5.1 ip BTZ pla bon rms PK.docx – *Final in vivo study plan as executed*
Attached File 5.2 Bortezomib study sheet.docx – *Submitted in vivo study digital data collection form (DCF)*
Attached File 5.3 Bortezomib Screening Plasma Tumor PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*

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